bio-protocol Preprint

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Quantitative real-time PCR (RT-qPCR) following BAF knockdown

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An abbreviated version of this protocol was published in Science in Aug 2020

BAF restricts cGAS on nuclear DNA to prevent innate immune activation

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Detailed protocol

BAF knockdown

Day 1

1. Plate HeLa cells in 6-well-plate at a density of 125 000 cells / well.
2. Cells are cultivated at 37°C and 5% CO_2.

Day 2

1. Cells were transfected with siRNAs using Lipofectamine®RNAimax reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Per well:
    - Tube A: mix 125µL of OptiMEM with 3.5µL of Lipofectamine®RNAimax reagent.
    - Tube B: mix 125µL of OptiMEM with 2µL of siRNA [initial concentration 20µM].
    - Mix tube A and B and incubate for 5 minutes.
2. Add 250µL of mixture on the cells.
3. Cells are added back in the incubator at 37°C and 5% CO_2.
4. Exchange the medium to fresh DMEM (+10% FBS, + Pen/Strep, +Glutamine) 6 h after transfection.
5. Cells are added back in the incubator at 37°C and 5% CO_2.

Day 5 (72h after transfection)

1. Remove media and wash cells with 2mL of PBS.
2. Remove PBS and add 350µL of RLT buffer (Qiagen, 79216).
3. Freeze the plate at -80°C or extract immediately RNA using the Qiagen RNeasy mini kit.

RNA extraction and cDNA production

1. Add 350µL of 70% Ethanol to the lysate and mix well by pipetting. Do not centrifuge and proceed immediately to step 2.
2. Transfer 700µL of the sample, including any precipitate to an RNeasy Mini spin column placed in a 2mL collection tube. Close the lid and centrifuge for 30 s at >8000 x g. Discard the flow-through.
3. Add 700µL buffer RW1 to the RNeasy spin column. Close the lid and centrifuge for 30 s at >8000 x g. Discard the flow-through.
4. Add 500µL Buffer RPE to the RNeasy spin column. Close the lid and centrifuge for 30 s at >8000 x g. Discard the flow-through.
5. Add 500µL Buffer RPE to the RNeasy spin column. Close the lid and centrifuge for 3 min at full speed. Discard the flow-through.
6. Place the RNeasy spin column in an empty 2ml collection tube. Centrifuge at full speed for 1min to dry the membrane.
7. Place the RNeasy spin column in a new 1.5mL collection tube (eppendorf 1.5ml). Add 30µL RNase-free water directly to the spin column membrane. Close the lid and centrifuge for 1 min at >8000 x g to elute the RNA.
8. RNA concentration is read on the TECAN reader.

DNAse treatment :

1. Mix:
    - 1µL DNase (Thermo Fisher Scientific, EN0521)
    - 1µl DNAse I buffer + $MgCl_2$ 10x (Thermo Fisher Scientific , B43)
2. Incubation 37°C for 30 min.
3. Add 1µL EDTA and incubate for 10 min at 60°C.

- 0.25µL RiboLock (#EO0381)
- 500 ng of RNA DNase treated
- Up to 10µL H2O

Reverse transcription (cDNA production) :

1. Mix :
    - 2µL of RT buffer
    - 0.5µL of Oligo dT [10 µM]
    - 1µL of dNTP mix (10 mM each)
    - 0.25µL of Ribolock (#EO0381)
    - 0.5µL of RevertAid Reverse Transcriptase (Thermo Fisher Scientific, EP0442)
    - 5.75 µl RNA sample (DNase treated)
2. Incubate at 42°C for 1 hour
3. Incubate at 70°C for 10 minutes.

Real-time PCR :

1. Add 40µL H_2O to the reverse transcription product.
2. In a 384-well plate for qPCR, mix :
    - 7.5µL of SYBR Green
    - 0.45µL of Primer Forward 10µM
    - 0.45µL of Primer Reverse 10µM
    - 4.1µL of Ultrapure water
    - 2.5µL of diluted reverse transcription product.
3. Run on a Real-time PCR machine (example : Thermo Fischer QuantStudio 5).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Guey, B. , Wischnewski, M. and Ablasser, A. (2021). Quantitative real-time PCR (RT-qPCR) following BAF knockdown. Bio-protocol Preprint. bio-protocol.org/prep1150.
2. Guey, B., Wischnewski, M., Decout, A., Makasheva, K., Kaynak, M., Sakar, M. S., Fierz, B. and Ablasser, A.(2020). BAF restricts cGAS on nuclear DNA to prevent innate immune activation . Science 369(6505). DOI: 10.1126/science.aaw6421

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